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Identification of an androgen-repressed mRNA in rat ventral prostate as coding for sulphated glycoprotein 2 by cDNA cloning and sequence analysis

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The concentrations of a small number of mRNAs in the rat ventral prostate increase after castration and then decrease upon androgen treatment. Since the repression of specific gene expression may be important in the regulation of organ growth, we have cloned a cDNA for an androgen-repressed mRNA, the concentration of which increased 17-fold 4 days after castration, and this increase was reversed rapidly by androgen treatment. By sequence analysis the androgen-repressed mRNA was identified as that coding for sulphated glycoprotein 2.

INTRODUCTION

Castration leads to the rapid involution of the ventral prostate of rats, since this organ requires adequate concentrations of androgens to maintain organ functions [1,2]. The precise mechanism of prostate involution has not been determined, but may be the result of both the loss of androgen-dependent synthetic activity [3–5] and an increase in certain degradative enzymes upon castration [6–8]. An increase in degradative activity is consistent with the cell death and regression that occur during involution [1,2]. Involution can be partly blocked with inhibitors of protein synthesis [9,10], suggesting that castration induces the synthesis of proteins involved in tissue remodelling. The cellular content of several proteins, whose functions are unknown [11,12], and the concentration of certain mRNAs [13–16] increase in the rat ventral prostate after castration. We have identified one of these androgen-repressed mRNAs as the mRNA for the Yb_1 subunit of glutathione *S*-transferase [15]. To characterize further the castration-induced gene response in the rat ventral prostate, we have used cDNA cloning and sequencing to identify an androgen-repressed mRNA as the mRNA for sulphated glycoprotein 2 (SGP-2) [17].

EXPERIMENTAL

Materials

Nick translation and sequencing kits, [α-^{32}P]dCTP (400–3000 Ci/mmol) and [α-[^{35}S]thio]dATP (1000 Ci/mmol) were from Amersham Corp., Arlington Heights, IL, U.S.A. *Escherichia coli* XLI-Blue was a product of Stratagene, La Jolla, CA, U.S.A. Nitrocellulose (0.45 μm pore size) was obtained from Schleicher and Schuell, Keene, NH, U.S.A.

Treatment of animals

Male and female rats of Sprague–Dawley strain, 90–120 days old, were used for experiments. Rats were castrated by the scrotal route or ovariectomized while under diethyl ether anaesthesia. When indicated, rats were given daily subcutaneous injections of 5 mg of DHT in sesame oil. Rats were killed by cervical dislocation.

Methods

Isolation of total RNA and polyadenylated RNA and analysis of the DNA content of guanidinium thiocyanate homogenates were as described elsewhere [13]. A cDNA library was constructed from polyadenylated RNA isolated from the ventral prostate of rats 3 days after castration. Double-stranded cDNA was synthesized [18], methylated at *Eco*RI sites, ligated to *Eco*RI linkers, digested with *Eco*RI, ligated to *Eco*RI-digested and dephosphorylated plasmid pUC13 and used to transform [19] *E. coli* XLI-Blue. Bacteria were selected on plates containing ampicillin, and clones with inserts were identified by using 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside and isopropyl β-D-thiogalactopyranoside [20]. Plasmids from individual clones were isolated [21] and sized by agarose-gel electrophoresis [20]. Plasmids, with inserts greater than 700 bp, were tested by dot hybridization [13] with ventral-prostate RNA from normal rats and from rats 1 to 5 days after castration, to identify clones for androgen-repressed mRNA. Control experiments with plasmid pUC13 were routinely performed and no significant hybridization was seen. The library was rescreened by hybridization *in situ* [20] with a labelled cDNA insert. Northern-blot analyses were performed in accordance with Maniatis *et al.* [20]. Double-stranded plasmid DNA was sequenced by the dideoxy chain termination method [22] as described by Hattori & Sakaki [21].

Computer sequence analysis

For sequence comparisons we used the National Biomedical Research Foundation/Protein Identification Resource protein sequence data base (version 16.0) and the GenBank data base (release 55.0; Intelligenetics). The computer programs were run on a VAX 11/750 computer from Digital Equipment Corp.

Abbreviations used: SGP-2, sulphated glycoprotein 2; DHT, 5α-dihydrotestosterone, 17β-hydroxy-5α-androstan-3-one.

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The sequence data for pSB28 have been submitted to the EMBL/GenBank Data Libraries under the accession number X13231.

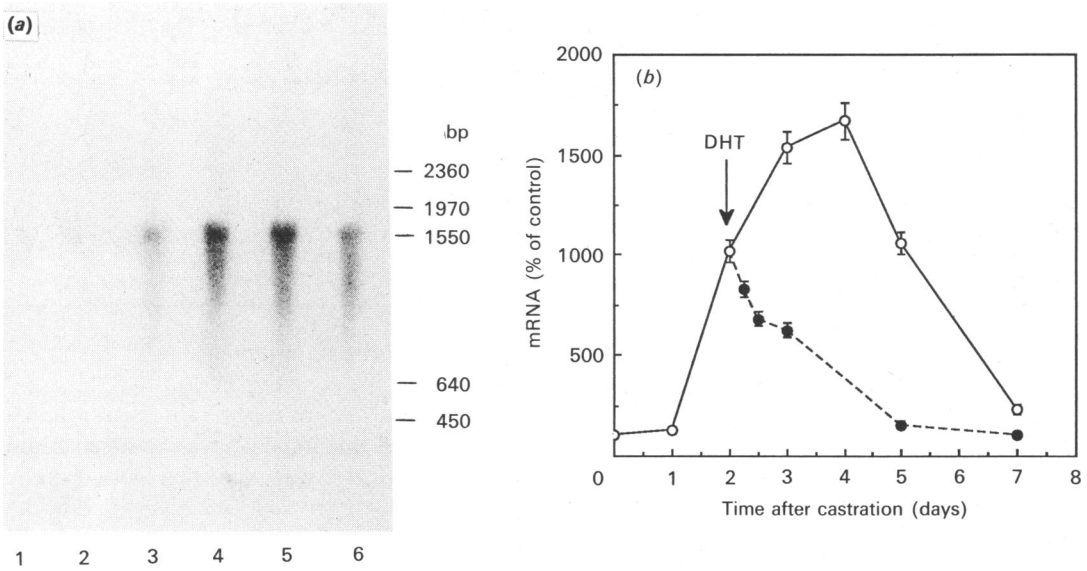

Fig. 1. Effect of castration and androgen treatment on the amount of mRNA hybridizing to pSB5 cDNA

(*a*) Total RNA (10 μg) isolated from the ventral prostate of normal rats (lane 1) or from rats 1–5 days after castration (lanes 2–6 respectively) was separated on a 1% agarose gel containing formaldehyde and then transferred to nitrocellulose. Hybridization was performed with a ^{32}P-labelled nick-translated cDNA insert (776 bp) of pSB5 that corresponds to nucleotide residues 910–1686 of pSB28. (*b*) Total RNA was prepared from the ventral prostate of normal rats or from rats 1–7 days after castration (○) and from rats given daily subcutaneous injections of DHT 2 days after castration (●). The amount of androgen-repressed mRNA hybridizing to pSB5 cDNA was analysed by dot-blot hybridization. Hybridization was performed as in (*a*). Radioactivity in individual dots was measured by liquid-scintillation counting and normalized per unit amount of DNA in tissue homogenates. The concentration of the mRNA in the ventral prostate of normal rats was set at 100%. Values are the means ± S.E.M. from four experiments in which organs from three to 20 rats were pooled for RNA isolation.

RESULTS AND DISCUSSION

Previously we have shown that four translation products with M_r values of 49000, 46000, 37000 and 29000 were the predominant products of translation *in vitro* of rat ventral-prostate RNA isolated from rats 3 days after castration [13]. A plasmid cDNA library was prepared from ventral-prostate mRNA, isolated from rats 3 days after castration, and randomly picked clones containing cDNA were screened by hybridization to ventral-prostate RNA from normal and castrated rats. A clone, pSB5, showed greater hybridization to RNA from castrated rats than to that from normal rats, and contained a cDNA insert of 776 bp. The cDNA insert in pSB5 hybridized to a 1800 bp RNA on Northern-blot analysis of ventral-prostate RNA from normal and castrated rats. The intensity of the 1800 bp band reached a maximum with RNA from rats 3–4 days after castration (Fig. 1*a*). On longer exposures an 1800 bp band was also visible in the Northern-blot analysis of RNA from normal rats or from rats castrated 1 day previously. When the mRNA concentration was normalized per unit of DNA to account for possible changes in RNA concentration and cell number after castration [13] we found that the amount of RNA (per unit of DNA) hybridizing to pSB5 increased 17-fold 4 days after castration (Fig. 1*b*). A rapid decrease in the concentration of this mRNA was observed within 6 h after a single injection of DHT into rats castrated 2 days previously. Subsequent daily injections of DHT decreased the concentration of this mRNA to normal values within 2–3 days.

Since the insert in pSB5 was too small to contain a full-length copy of a 1800 bp mRNA, we rescreened the cDNA library by hybridization *in situ* with the insert of PSB5. One clone, pSB28, that hybridized to the cDNA insert of pSB5 contained an insert of 1721 bp. An open reading frame contained 1341 bp, and a putative initiation methionine residue [17] within a consensus translation initiation sequence [23] preceded a sequence that is typical of a leader peptide. By using a computer search of sequence data bases, we found that the sequence of pSB28 cDNA was essentially identical with the cDNA sequence of SGP-2, a secretory product of Sertoli cells [17]. A single base difference was found in the coding region at position 559 (G in pSB28 and C in SGP-2), changing the encoded histidine in SGP-2 to an aspartic acid residue in pSB28. In the 3′-non-coding region, one nucleotide residue (C) at position 1432 and six nucleotide residues (GCCGAT) at positions 1563–1568 in pSB28 were not present in the reported sequence of SGP-2 cDNA. The additional bases at positions 1563–1568 in pSB28 cDNA came just before the poly(A) tail; therefore this difference may be due to a variation in processing of the 3′-end of the mRNA before addition of the poly(A) tail. The 5′-end of pSB28 cDNA differed from the reported sequence of SGP-2 cDNA between positions −61 and −29 (−61: CAGGAGGAGCGCACTGGAGCCAA-GCCGCAGACG: −29). The published sequence of SGP-2 cDNA [17] had an additional 237 bp beyond the 5′-end of pSB28 cDNA. These differences in the 5′-non-coding region of pSB28 and SGP-2 cDNA may represent cloning artifacts or perhaps tissue-specific processing of the RNA. The cDNA in pSB5 was also sequenced and

Table 1. Comparison of the amounts of the mRNA hybridizing to pSB5 cDNA in various organs of male (M) and female (F) rats

Total organ RNA was isolated from normal rats or from rats castrated 4 days previously. The amount of the mRNA hybridizing to pSB5 cDNA was measured by dot hybridization as described in Fig. 1 legend. The castrated/normal (C/N) ratio was calculated by dividing c.p.m./μg of DNA for tissues from normal rats by c.p.m./μg of DNA for tissues from castrated rats. Values are the means $\pm$ S.E.M. from three to seven experiments in which organs from five to 20 rats were pooled for RNA isolation. Abbreviation: N.D., not determined.

		Bound ^{32}P-labelled pSB5 cDNA		
		Amount bound (c.p.m./μg of DNA)		
Organ	Sex	Normal	Castrated	C/N ratio
Ventral prostate	M	74.5 $\pm$ 8.8	1243.0 $\pm$ 125.0	16.7
Seminal vesicle	M	174.0 $\pm$ 6.6	388.9 $\pm$ 29.2	2.2
Dorsal–lateral prostate	M	169.2 $\pm$ 11.7	345.9 $\pm$ 42.0	2.0
Liver	M	229.5 $\pm$ 12.9	348.8 $\pm$ 36.0	1.5
Kidney	M	10.1 $\pm$ 1.4	10.9 $\pm$ 1.3	1.1
Brain	M	100.7 $\pm$ 7.7	107.7 $\pm$ 10.7	1.1
Heart	M	122.5 $\pm$ 23.5	85.9 $\pm$ 18.2	0.7
Coagulating gland	M	15.2 $\pm$ 1.9	8.1 $\pm$ 2.3	0.5
Submaxillary gland	M	45.2 $\pm$ 2.6	23.4 $\pm$ 1.8	0.5
Testis	M	337.5 $\pm$ 15.1	N.D.	N.D.
Epididymis	M	187.5 $\pm$ 7.2	N.D.	N.D.
Lung	M	4.0 $\pm$ 0.6	N.D.	N.D.
Skeletal muscle*	M	2.4 $\pm$ 0.5	N.D.	N.D.
Spleen	M	1.5 $\pm$ 0.2	N.D.	N.D.
Uterus	F	8.4 $\pm$ 1.3	22.5 $\pm$ 0.6	2.7
Liver	F	112.5 $\pm$ 9.9	156.3 $\pm$ 11.4	1.4
Heart	F	81.3 $\pm$ 5.3	88.6 $\pm$ 8.6	1.1
Kidney	F	13.0 $\pm$ 1.8	13.8 $\pm$ 1.1	1.1
Brain	F	73.4 $\pm$ 7.9	72.0 $\pm$ 7.9	1.0
Ovary	F	25.3 $\pm$ 0.9	N.D.	N.D.

* Quadriceps femoris muscle.

corresponded to nucleotide residues 910–1686 of pSB28.

The open reading frame of pSB28/SGP-2 codes for a protein containing 447 amino acid residues with a deduced M_r of 51382. Hybrid-arrest translation of rat ventral-prostate mRNA, isolated from rats 3 days after castration, with the cDNA insert of pSB28, arrested the translation *in vitro* of a 49000-M_r product (results not shown), which is similar to the predicted size of SGP-2, and corresponds to the largest of the products of translation *in vitro* of ventral-prostate mRNA from castrated rats [13].

By dot hybridization we found that RNAs from several organs of male and female rats contained RNA hybridizing to pSB5 cDNA (Table 1). Northern-blot analysis of RNA from these organs showed hybridization only to an RNA of 1800 bp (results not shown). The amounts of RNA hybridizing to pSB5 cDNA from the ventral prostate, uterus, seminal vesicle, dorsal–lateral prostate and liver increased after castration, but decreased in male heart, submaxillary gland and coagulating gland. The relative amount of RNA hybridizing to pSB5 cDNA from various organs was similar to that reported for pSGP-2 cDNA [17]; testis, epididymis and liver contained the highest concentrations of pSB28 mRNA.

Tenniswood and co-workers [14] have described an androgen-repressed mRNA from rat ventral prostate with some characteristics similar to those we describe here for SGP-2 mRNA; however, the identity of their mRNA was not known. They did not detect the mRNA in liver, kidney or seminal vesicle before or after castration. We have found SGP-2 mRNA in each of these tissues, and rat liver, in particular, had high concentrations of SGP-2 mRNA. Therefore the androgen-repressed mRNA characterized by Tenniswood and co-workers [14] may not be SGP-2 mRNA.

In testis SGP-2 is a major glycoprotein secreted by Sertoli cells [24]. The protein associates with the distal portion of mature spermatozoa [25], suggestng a role for SGP-2 in sperm function. We do not know if SGP-2 is a component of prostatic fluid, but the ventral prostate and other accessory reproductive organs may secrete SGP-2 into seminal fluid, where it may have an effect on sperm.

The mechanism and role of androgen-repressed gene expression in the rat ventral prostate is not known. Androgens and other steroids have negative effects on gene expression in other systems [26]. It has been proposed that glucocorticoids interfere with transcription of the gene for the α-subunit of glycoprotein hormones (which are also negatively regulated by androgens) by competition of the glucocorticoid receptor for gene regulatory sites normally occupied by transcription factors responsive to cyclic AMP [27]. A similar competition for transcriptional regulatory sites by the androgen receptor may be responsible for the effects of androgens

on the concentrations of mRNAs for the Yb_1 subunit of glutathione *S*-transferase [15] and SGP-2.

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